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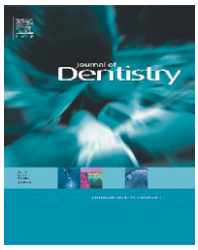

Effect of a 10% carbamide peroxide on wear resistance of enamel and dentine: *In situ* study

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ABSTRACT

Objectives: This triple-blind, 2 × 2 crossover *in situ* study, was undertaken to verify whether the wear resistance of enamel and root dentine would be affected by bleaching with a 10% carbamide peroxide agent and a placebo agent.

Methods: Thirty slabs of each substrate (2 mm × 3 mm × 2 mm) were selected for each phase, after flattening and polishing procedures and microhardness test. After a 7-day lead-in period, one specimen of each substrate was randomly bonded on the facial surface of each one of 30 subject's upper second premolars. The volunteers received instructions on how to perform toothbrushing and application of gel in the tray. Fifteen volunteers bleached their maxillary arch with a 10% carbamide peroxide bleaching agent for a 2-week period, while the remainders used a placebo agent. After a 1-week washout period, a new set of enamel and root dentine slabs were bonded to the premolars and volunteers were crossed over to the alternate agent for 14 days. The resistance of enamel and root dentine to wear following bleaching, toothbrushing and intraoral exposure was measured with a profilometer, using reference areas.

Results: For enamel, ANOVA did not demonstrate significant difference between wear provided by placebo and bleaching agent ($p = 0.3713$), but higher wear depth was observed for bleached root dentine ($p = 0.0346$).

Conclusions: While overnight bleaching caused no alteration in wear resistance of enamel, root dentine showed increased tissue loss.

1. Introduction

Appearance of the teeth is of great importance to patients that seek esthetic treatments related to dental discoloration. Among treatments, tooth bleaching has attracted the interest of patients and dentists because it represents a non-invasive option and is relatively simple to carry out.[1] At-home bleaching technique, in particular, received worldwide acceptance when described in 1989 by Haywood and Heymann.[2] Ten percent carbamide peroxide is the bleaching agent most used in this technique[2] as it is considered safe and effective.[3] As whitening of vital teeth generally involves direct and frequent contact of the bleaching agent with the outer enamel surface and sometimes with dentine for an extensive period of time, *in vitro* studies have evaluated the effects of carbamide peroxide on dental hard tissues. Changes have been observed in surface texture,[4–8] mineral content,[9–12] chemical composition,[6,13,14] and loss by toothbrushing abrasion.[15]

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Additionally, some *in situ* studies, that represent an intermediate stage between *in vitro* studies and clinical trials,[16] have investigated the effects of bleaching treatment on microhardness of dental enamel[13,17–19] and dentine.[18,20] Surface morphology,[13,21] roughness[21] and calcium content in enamel[13] have also been investigated. However, the effect of whitening agents on wear of enamel and dentine, using an *in situ* model, has still not been evaluated.

Considering that there are *in vitro* reports showing that bleaching of enamel with 10% carbamide peroxide can cause demineralization up to 50 μm below the surface[10] or even up to 150 μm,[9] it could be assumed that the dental substrate could be less resistance to wear processes. In fact, Wiegand et al.[15] have already shown that bleaching increase the substrate loss by toothbrushing. Nevertheless, no *in situ* study validated the alteration and the increase of wear of enamel and dentine.

As in *in situ* studies the remineralization potential of human saliva can minimize the adverse effects of bleaching agents on enamel and dentine,[18] there are doubts if the bleaching gel may increase the susceptibility of dental tissues to wear.

Therefore, this *in situ* study was conducted to evaluate the effect of bleaching with 10% carbamide peroxide agent on wear of enamel and root dentine.

2. Materials and methods

2.1. Ethical aspects and volunteers

The protocol of this study was reviewed and approved by the Ethics Committee of the School of Dentistry of Ribeirão Preto, USP (process no. 2004.1.834.58.6). Thirty volunteers (28 females and 2 males, aged 19–43 years) who fulfilled the inclusion criteria (normal saliva flow, absence of dental caries and/or periodontal disease, willing to perform bleaching treatment following the research schedule) without violating the exclusion criteria (use of orthodontic appliances, presence of fixed or removable denture, pregnant or nursing women, smokers and dentine sensitivity) took part in this study after signing an informed, written consent (Resolution no. 196 from National Health Council, Brazil, 1996).

2.2. Experimental design

This triple-blind study followed a two-period crossover design. Each phase lasted 14 days and a washout period of 1 week between each phase. The factor under study was bleaching treatment at two levels: a 10% carbamide peroxide and a placebo agent, as listed in Table 1. Volunteers were randomly divided into two groups of 15, and each group received the bleaching or the placebo agent in different sequence, in two distinct periods (bleaching agent-placebo; placebo-bleaching agent). The experimental units consisted of 60 bovine enamel slabs and 60 bovine root dentine slabs, randomly assigned to the 30 volunteers (one enamel slab and one dentine slab per phase). Each volunteer was considered a statistical block. The response variable was the wear depth (μm) evaluated profilometricaly. The experimental set-up of this study is shown in Fig. 1.

2.3. Preparation of dental slabs

Sixty freshly extracted bovine incisors were cleaned to remove tissue remnants and stored in 0.1% thymol (pH = 7.0). The roots were separated from their crowns in the cementoenamel junction using a low speed water-cooled diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA). Enamel slabs (3 mm × 2 mm × 2 mm) were obtained from the middle third of the labial surface, while dentine slabs (3 mm × 2 mm × 2 mm) were cut from the cervical third of the root surface. Dental slabs were fixed with stick wax in acrylic resin cylinders and the upper surface of the samples was then flattened and serially polished with 400-, 600- and 1200-grit Al_2O_3-abrasive papers and with 0.3- and 0.05-μm alumina polishing suspensions (Alpha and Gamma Micropolish, Buehler, Lake Bluff, IL, USA) on cloths in a water-cooled mechanical grinder (Struers A/S, Rodovre, Denmark). To remove polishing debris, specimens were placed in an ultrasonic cleaner (T1440D, Odontobrás Ltda., Ribeirão Preto, SP, Brazil) with distilled water for 10 min. A stereomicroscope (Nikon 88286, Tokyo, Japan) at 40× magnification was employed to select and discard samples that presented pits or cracks. Enamel and root dentine sections were then gas sterilized (ethylene oxide).

2.4. Selection of enamel and root dentine slabs

After sterilization, microhardness measurements were performed on sound substrates, through a Knoop indenter (HMV-2, Shimadzu, Kyoto, Japan), under a 50-g load for enamel and 25-g load for root dentine applied for 30 and 10 s, respectively. Four indentations, located 500 μm from the margin of the dental slab and 250 μm apart, were made and the surface microhardness (SMH) was calculated for each specimen. A total of 60 slabs of enamel and 60 slabs of root dentine were selected based on the mean values obtained for each dental substrate (enamel and root dentine).

Table 1 – Bleaching treatments tested, their application protocol and pH.

Agent	Code	Basic composition[ψ]	Application protocol	pH	Batch #
Whiteness Perfect (10% carbamide peroxide)	CP10%	10% carbamide peroxide, neutralized carbopol, glycerol and distilled water	8-h daily application, for 14 days	6.14[a]	16FEV06
Placebo (Whiteness Perfect)	PLA	Neutralized carbopol, glycerol and distilled water	8-h daily application, for 14 days	6.28[a]	16FEV06

[ψ] As provided by the manufacturer (FGM, Joinville, SC, Brazil).
[a] Measured with mPA Tecnopon (mPA Tecnopon, MS Tecnopon Equipamentos Especiais Ltda., Piracicaba, SP, Brazil).

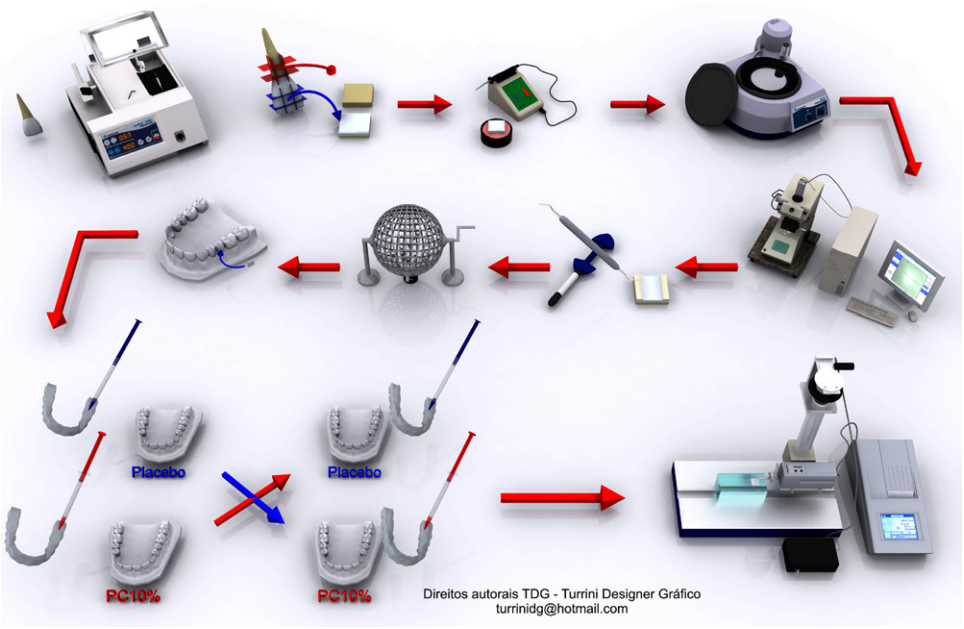

Fig. 1 – Schematic drawing of the experimental set-up: using a low speed water-cooled diamond saw, bovine enamel and root dentine slabs (3 mm× 2 mm × 2 mm) were obtained. Samples were fixed with stick wax in acrylic resin cylinders, and ground flat and polished in a water-cooled mechanical grinder. Slabs were pretested using a microhardness tester. Two-thirds of the surface area of specimens was covered with resin composite. The specimens were randomly fixed on the facial surface of the maxillary second premolars of 30 volunteers. The volunteers were divided into 2 groups of 15: in the first experimental phase, the Group I received the bleaching agent and the Group II received the placebo, for a period of 2 weeks. In the second phase, after a washout of 1 week, the Group I received the placebo and the Group II received the bleaching agent, characterizing a 2 × 2 crossover study. The composite resin was removed from the specimens and wear depth was assessed in profilometer, in relation to the reference area.

2.5. *Preparation of dental slabs for the experimental phase*

In order to ensure the presence of reference surfaces (area unexposed to the bleaching agent and to the oral cavity and unbrushed) when measuring the depth of the abrasion grooves, a thin layer of composite resin was applied over the two ends of surface of the specimens, based on the description of Amaechi and Higham,[22] leaving a window of 1 mm × 2 mm × 2 mm in their central area. The resin composite (Herculite XRV, Kerr, Emigsville, PA, USA) was light-cured for 40 s and polished with aluminum oxide discs (Sof-Lex, Pop-On, 3M/Espe, St. Paul, MN, USA). Specimens were stored at 37 ± 0.5 °C in 100% relative humidity.

2.6. *Tray preparation*

Upper and lower dental arch impressions were taken with alginate using a stock tray and stone cast molds were made. On the molds, vestibular reservoirs were generated with one coat of nail varnish on all teeth, except on the second premolars where reservoirs (3 mm × 4 mm × 3 mm) were prepared with resin composite corresponding to the specimen that would be bonded on the volunteers' teeth. Two trays were manufactured for each volunteer using a 1-mm thick flexible ethyl vinyl acetate polymer in a vacuum tray-forming machine (P7/Bio-Art Equip Odontológicos Ltda., São Carlos, SP, Brazil).

2.7. *Preparing the volunteers for the experimental phase*

In the pre-experimental period or run-in phase, which lasted 1 week, each volunteer received a toothbrush (Oral-B Indicator Plus 35, Gillette do Brasil Ltda., Manaus, AM, Brazil) and a fluoridated dentifrice (Colgate Cavity Protection, Colgate-Palmolive Ltda., Osasco, SP, Brazil, AO).

A complete prophilaxis was performed on each patient and the initial color of his/her teeth was determined by Vita scale (Wilcos do Brasil Indústria e Comércio Ltda., Petrópolis, RJ, Brazil).

2.8. *Experimental phase*

The 30 volunteers were randomly divided into 2 groups of 15. First, the tray was tested, and when necessary, adjustments were performed. After, two dental specimens – one of enamel and one of root dentine – were randomly bonded to the vestibular surfaces of the superior second premolars (if absent, the upper first premolars) of each patient. The base of the specimens and the area corresponding to the place to fix it in the labial surface of the superior second premolar were acid etched with phosphoric acid. In the sequence, an

adhesive system (Single Bond, 3M/Espe, St. Paul, MN, USA) and a resin cement (Rely-X, 3M/Espe, St. Paul, MN, USA) were applied following the manufacturer's instructions. The slab was positioned on the correct area, the excess of the cement was removed and light-cured. The volunteers were instructed on how the bleaching treatment would be performed, the manner to apply the bleaching gel (Whiteness Perfect 10%, FGM, Joinville, SC, Brazil) or placebo gel (Whiteness Perfect, FGM, Joinville, SC, Brazil) in tray, how to clean the tray after removing it from the mouth and keep it in a container provided. Moreover, with the aim to standardize the tooth-brushing technique, instructions were given to the volunteers on how to brush their teeth and bonded specimens. In the experimental Phase 1, Group 1 applied the bleaching agent, while Group 2 applied placebo agent in the tray and wore it overnight for about 8 h. The volunteers were blind as to which agent they were using.

After the bleaching period (14 days), the specimens were carefully removed with orthodontic pliers and the residual cement was removed with resin polishing carbide burs (Jet, Beavers Dental, ON, Canada) and aluminum oxide discs (Sof-Lex, Pop-On, 3M/Espe, St. Paul, MN, USA).

Volunteers were submitted to a washout period of 1 week with the aim to eliminate the residual effects of the treatment previously applied. Then, they received new toothbrushes and dentifrices and the dental hygiene technique was reinforced.

In the experimental Phase 2, a new set of dental slabs were randomly bonded in the same way as that used for experimental Phase 1. New trays were distributed to the volunteers to eliminate any possible residues left by the previously applied agent. In this phase, the Group 1 applied the placebo agent, while Group 2 used bleaching agent for another 2 weeks. The specimens were removed in the same manner that in the Phase 1. After finishing the experimental Phase 2, the volunteers began the bleaching treatment in the mandibular arch. At the end of the bleaching treatment, photographs were taken and some restorations were replaced for esthetic reason.

2.9. *Wear depth measurements*

To determine wear, the composite resin was removed from the specimens. As the acid etching/adhesive system was not used, the composite resin was carefully and gently detached from the enamel and root dentine surface, exposing the untreated reference areas. The specimens were cleaned ultrasonically for 10 min. The wear depth, on both tissues, was measured using a profilometer (Surfcorder SE-1700, Kosaka Corp., Tokyo, Japan) equipped with a diamond stylus of 2 μm radius. Five profilometric traces at a constant speed of 0.1 mm/s and a load of 0.7 mN, perpendicular to the brushing direction, were performed for each specimen. The average of these five measurements was used as the wear depth value for each specimen.

2.10. *Statistical analysis*

Period and *carryover* effects were verified by paired *t*-tests. The data were statistically evaluated using two-way analyses of variance (ANOVA) with a significance level of 5%. Tukey's test was applied where significant differences were detected. The software Statgraphics Centurion XV (Statgraphics Plus Software, Manugistics, Rockville, MD, USA) was used to perform the statistical analyses.

Table 2 – Means (standard deviations) of surface wear (μm) of enamel and root dentine exposed to 10% carbamide peroxide and to placebo agents.

Substrate	Enamel	Root dentin
CP 10%	1.78 (0.83)	2.67 (1.31)
Placebo	1.59 (0.83)	2.08 (1.07)

Means connected by brackets did not differ from one another ($\alpha = 0.05$; l.s.d. = 0.55) for the same dental substrate.

3. Results

The paired *t*-tests verified that the effects of period ($p = 0.99$ for enamel and $p = 0.15$ for root dentine) and carryover ($p = 0.44$ for enamel and $p = 0.36$ for root dentine) were not statistically significant. Table 2 shows the means and standard deviations of enamel and root dentine wear.

For enamel, ANOVA did not demonstrate significant difference between wear provided by placebo and bleaching agent ($p = 0.3713$), but higher wear depth was observed for bleached root dentine ($p = 0.0346$).

4. Discussion

Different protocols of *in situ* studies have been used to assess the effects of bleaching treatment on dental hard tissues. Among the described protocols in the literature, most of them adopted the use of a removable palatal appliance containing slabs of enamel[13,17,19] or dentine.[20,23] Some disadvantages about this protocol may be identified such as the difficulty to ensure that all volunteers followed it suitably; the fact that did not represent the erosive and abrasive challenge that occurs in the oral cavity, as the appliance is removed during meals and hygiene procedures and the abrasive effect that the tongue can exert on dental tissues, increasing the overall loss of tooth substance.[24] Moreover, another important constraint about bleaching experiments in which removable palatal devices were used[17,19,20] is that it is impossible to reject the probability of contact of the bleaching gel with the control specimens or, to avoid the residual effect of bleaching gel in the control group, considering that the slabs were present in the same device. In other researches, control specimens were removed from appliance and kept in artificial saliva, while experimental specimens were bleached in the mouth;[23] or the bleaching treatment was performed outside the oral cavity and then placed in the mouth of volunteers.[13]

In the present study, an *in situ* model was chosen to simulate the bleaching treatment, as closely as possible of the clinical situation, avoiding these above-mentioned problems. The adopted protocol was similar to Basting et al.,[18] in which

specimens of enamel and root dentine were fixed on the facial surface of the maxillary second premolars. Another important aspect related to the current study was the crossover experimental design, in which each volunteer performed the treatment with the bleaching and placebo gel, in different periods, with a washout of 1 week to eliminate the residual effect between the treatments. Thus, each volunteer was considered one complete statistical block, eliminating the different habits among them,[25] such as diet, toothbrushing force, and biological factors including flow rate, buffering capacity and composition of saliva.

Ten percent carbamide peroxide agent was used to represent the gel most commonly used in home bleaching. In this research, special care was taken in the choice of control group, represented by a placebo, which was prepared by the same industry of the bleaching agent. Thus, the placebo presented exactly the same appearance, color, flavor, consistency and composition, with exception of the 10% carbamide peroxide. The protocol of bleaching treatment was performed according to manufacturer's instructions.

A platform of composite was constructed on the two ends of the selected slabs and then fixed on the teeth of the volunteers, with the aim of serving as a reference area to allow latter evaluation of dental loss, and also providing protection of the exposed area against the abrasion from soft tissues, as proposed by Amaechi and Higham.[22] Slabs of enamel and root dentine used in the present experiment were obtained from bovine teeth, due to the fact that they present similar chemical composition to human teeth.[26] Moreover, bovine teeth are easily obtained and manipulated, because of their large size.

In terms of wear, few studies in the literature have evaluated the effects of whitening agents on dental substrates. Among the different methods used to assess the loss of dental hard tissue, profilometry was chosen because it is a method of high precision[27] and permits the measurements of wear in a relatively simple way.[28]

With respect to enamel, the result of the current study did not reveal a significant difference between the wear provided by 10% carbamide peroxide and the placebo. This finding corroborates a previous *in vitro* study in which 10% carbamide peroxide applied by the same period of this study did not affect the enamel wear produced by an oral wear simulator in comparison with untreated specimens.[29] However, enamel bleached with 10% carbamide peroxide and submitted to cycles of toothbrushing showed slightly greater wear than high concentration carbamide peroxide agents.[29] Despite of the fact that the bleached differed from the unbleached group, the magnitude of wear has been considered clinically irrelevant.[15] In fact, the mean value obtained was around 0.207 μm, wear considered very small compared to values observed in this *in situ* study (Table 2). The reports of Attin et al.[9] and Efeoglu et al.,[10] have demonstrated that the 10% carbamide peroxide are able to penetrate and diffuse through enamel. Thus, demineralization and reduction of subsurface microhardness have been observed up to 50 μm after 15-d bleaching period[10] and 150 μm below the surface of the enamel after 10-d bleaching period[9]. Possible explanations for these alterations are the time of application of the bleaching gel,[15] insufficient remineralizing period to repair subsurface microstructural defects[9] and the result of the uncontrolled reaction of the peroxide radical.[10] As a consequence of these surface and subsurface changes, enamel could be more susceptible to abrasive wear. However, due to the biological factors of saliva – flow, buffering capacity, acquired pellicle, composition – and to pH value of the bleaching agent does not affect the enamel,[30] the increase of wear was not verified in this study.

Some *in situ* investigations also are in agreement with the absence of difference between bleaching and control treatment in terms of microhardness,[13,17] micromorphology[13,21] and content of calcium.[13] In contrast with these findings, alterations on the microhardness[18] and surface roughness[21] have been observed, probably because of the prolonged contact between the bleaching gel and the dental tissue.[18,21] Moreover, the carbopol present in the placebo and bleaching agent may have caused changes in dental structure microhardness and in its mineral content.[21]

With regards to root dentine, the specimens exposed to the bleaching gel exhibited higher wear value than the placebo gel. This finding may be attributed to the composition and structure of this tissue,[31] which possess higher organic content than enamel,[26] besides to present higher porosity and solubility.[26,32] Other important aspect to consider is the critical pH for the root dentine which is in the range of 6.2 to 6.7.[32] In the current experiment, although the pH of both gels presented lower value than that established as critical for root dentine, the pH of the placebo was higher than the pH of the bleaching agent (Table 1). Thus, during demineralization, a loss of mineral from the tissue occurs and, together with brushing, may increase dentine surface wear. Changes on the dentine after the use of 10% carbamide peroxide were also observed on surface morphology,[8] microhardness[11,12] and mineral content[14] of *in vitro* studies and on the microhardness of an *in situ* study.[20] Probable explanations for these alterations are the low pH,[11,12] composition of the bleaching agents[12] and protocol of the bleaching treatment.[8,14] On the other hand, in some *in vitro* studies[9,33,34] there were no changes neither on the surface[34] and subsurface[9] microhardness nor on the morphology and surface roughness of dentine,[33] owing to the short-term regimens[33,34] and neutral pH of bleaching agents.[9] The *in situ* studies of Basting et al.,[18,21] also did not show significant difference on microhardness and surface roughness, probably due to the pH of bleaching agent used (6.7).

5. Conclusions

At-home bleaching performed overnight with 10% carbamide peroxide did not affect enamel wear, but caused higher wear rates in root dentine.

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